

## Comparative Aspects of the Clinical Hematology of Birds: A Review

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**Abstract:** Clinical hematology is examination of cellular, fluids of blood and a study of the tissue that form, store and circulate in the blood cell. Hematology disorder is approached by identifying the primary hematopoietic component that is affected: white blood cells, red blood cells and platelets or the coagulation system. Major abnormalities in hematology are quantitative in nature with either excessive or deficient production of one of the hematopoietic constituents. In birds blood samples collected for hematology analysis should be venous origin (wing and brachial vein). After staining leukocytes and erythrocytes counted using the eosinophil unopette, Natty Herrick's solution or by using an automated cell count. Avian erythrocytes are oval nucleated cells with immature form. As in other mammals the periphery of chicken contain granulocytes (heterophil, eosinophil and basophil) arise from a common precursor in the bone marrow, while lymphocytes and monocytes grouped under granulocytes since this cell do not have granule. The normal reference interval of these blood components vary in different avian species.

**Key words:** Hematology • Granulocytes • Agranulocytes • Birds

### INTRODUCTION

Clinical-hematology is the application of research on blood and the associated organ systems to the clinical treatment of patients with blood and organ disorders. This includes everything from managing patients with anemia to the treatment of patient with acquired blood born diseases [1].

Although the chicken has been used as the research animal model for establishing normal parameters for other avian species little information has been published on hematology of chicken and turkeys in a clinical setting [2].

The chicken and turkeys have recently become more popular as backyard "pets" and visits to the veterinary hospital are increasing. Most of the current information about routine hematologic parameters is extricated from clinical values established for *psittacines*. Although serology is the predominant method of disease monitoring in commercial poultry, examination of blood smears, bone marrow and clinical chemistry value is rarely done. The genetic, body size and production purpose of chicken and Turkeys is highly divergent and influence diseases resistance as well as production parameters [3].

Hematology disorders can be approached by indentifying the primarily *hematologic* components that is affected RBCS, WBC, *platelets* or the coagulation system. The major abnormalities in hematology are quantitative in nature, with either excessive or deficient production of one of the hematopoietic constituents (E.g. Leukemia, anemia or qualitative abnormalities also can be inherited (E.g. sickle cell disorders) or acquired [1].

Hematology includes not only the examination of cellular and fluids of blood but also includes a study of the tissues that form, store and circulate blood cells. The interpretation of avian blood cell provides many challenges; a veterinarian uses the result of hematology test to help determine the health of avian. These results are used in conjunction with history, physical examination and other finding [4]. Recognition of avian blood cells and interpretation of change require knowledge and experience [5]. Therefore the objectives of this Seminar Paper are to describe the normal morphology and function of cell in avian blood to interpret change in those cells and to review maturation sequence of avian blood cell and their reference interval.

**Collectoin of Blood Sample:** Blood collected from a bird should be of venous origin and in most birds it can be taken from the *basilic vein* (Wing or brachial vein) which cross the ventral surface of the humeral *redioulnar joint* (Elbow) immediately under the skin or the jugular vein usually the right which is larger than the left and caudal *tibial vein* (medial metatarsal which is located on the medial side of the *tibiotarsus* above the tarsal joint, sample can be collected from the most species of birds under manual restraint in the dorsal position [6].

The wing vein is superficial and easily visualized in adult birds and chicken and *poults* order than 1.5-2 weeks of age, both wing can be used and collection can be performed with or without assistance. To prevent flapping and kicking, control of unused wing and feet is essential. This is achieved by holding the bird in one arm with the birds feet focusing behind the upper arm towards the rear pinning the unused wining between the birds body and collectors trunk and out stretching the target wing with the holding arm. The birds head generally hongs down which has a calming effect. This method leaves the collector one hand free to collect the blood but also can be used when assisting other [2].

For highly aggressive birds (Fighting chickens), heavy birds (Tom turkey) and newly hatched birds, the right jugular vein is used. High aggressive birds often cannibalize their wing if there is blood spot or hematomas. The right jugular vein is more superficial than the left jugular vein and the hematoma formation is less than the wind veins puncture [6].

Cardiac puncture is used in a situation where birds will be euthanized immediately after the procedure and commonly used for assisting maternal antibody level in a newly hatched chicken. Due to the risk of damaging the lung and expectoration of blood, this technique must only be used by trained personnel if birds are not euthanized [2].

The average blood volume of most birds is approximately 10% body weight. Ten percent of this volume may be removed from circulation for testing (Or 1% of the bird's body weight) for example, up to 3ml may be collected from 300mg parrot [7].

**Preparation of Blood Smear:** Wipe all slides using a clean lint free cloth or tissue before use collect a small aliquot of blood from sample using a plain capillary tube and place small drop (Approximately 2 $\mu$ l) at one end of the slide about 45°C move the spreader backwards and touch gently the drop of blood, which will run across the edge of the slide. Push the spreader with a steady towards movement to create a uniform blood smear [6].

**Cell Counts and Staining:** Although anticoagulant, particularly heparin, can cause artifacts in blood smear, it is impractical to do blood smear immediately after blood collection [7]. Blood can be collected in a syringe created with anticoagulant, traditionally ethylene diaminetetra acetic acid (EDTA) for avian blood samples, or in an uncoated syringe with blood transferred to a coated collection tube although EDTA samples were minimal [8].

Transferring to coated tube allows more control of the ratio of the blood volume to anticoagulant; particularly in cases where small quantities are drawn. The standard two side technique can often result in a high number of smudge cells as some avian cell tend to be more susceptible to rupture than mammalian cells [9]. When using this technique, sufficient numbers of smears need to be produced to accommodate loss of imperfect smears. Two alternate methods can be used: place a small drop of blood on to a slide and place a cover slip over the drop horizontally remove the cover slip as the blood drops spreads [9]. This method sometimes results in overly thick viewing areas and granule shape can be difficult to ascertain put small drop of blood on a cover slip and use *cytospin* centrifuge to distribute the drop evenly around the cover slip [2].

Smears using all three methods need to be scanned to determining the percentages of smudge cells in relation to 100 counted cells. The traditional stains used for mammals can also be used for avian blood smears. Recently a new staining method specifically designed for avian cell has been described [10]. Using this method, white blood cell color differentiation and cellular identification can be performed in addition to size and shape [2].

Leukocyte counts using the eosinophilunopette 5877 or natty herrick's solution are considered the gold standard for counting avian samples [9]. Authomated systems, while useful in some situations such as determining the heterophil/lymphocyte ratio (H: L) are not accurate for differentiating all cell types [11]. Flow cytometry has been successfully used and has been shown to accurately separate lymphocyte from thrombocyte, but may not be practical in a clinical setting [12]. Manual counting cells from a blood smear is often inaccurate and is only recommended for situations where other methods are not available. Since some cell types can be preferentially lysed when using the two slide pushing technique, it is important to use only good quality smears counting the number of leukocytes observed in 2-4 high power fields each on at least five smears, count the number of leukocytes per high power field on at least 10 fields, average and multiply by 2000 [2].

Erythrocytes can be counted using on erythrocyte unopette method or by using on authomated cell counter. Both leukocytes and erythrocytes will be counted using the automated counters but the leukocyte numbers are small in comparison with the erythrocytes and do not skew erythrocyte counts. Packed cell volume (PCV) is determined using the micro *hematocrit* method. Hemoglobin can be determined using the same method used for mammalian species but he sample needs to be centrifuge, before obtaining the optical density to remove nuclei after cell lysis. Mean corpuscular hemoglobin (MCH), MCH concentration (MCHC) and mean corpuscular volume (MCV) can be calculated. Reticulocytes can be estimated after staining with new methylene blue (NMB). Residual *cytoplasmic* RNA stains basophilic with NMB. A reticulocyte count of approximately 3% can be seen in normal health chicken [2].

**Hematopoiesis:** *Hematopoiesis* is a complex two stage processes by which mature blood cell of distinct lineages are produced from *pluripotent* stem cell (HSC) during the entire life span. The first stage is represented by the differentiation of HSL from early mesoderm cell, the second stage is represented by further differentiation of the *multipotent* stem cell progenitors (HPC) that become committed to develop in to cell of different lineage, defined as burst forming unit (BFU) and colony stimulating factors [13].

Most research on ontogeny of the hematologic and lymphoid systems has been done in the chicken and quail frequently by using chicken quail *chimeras* [14]. *Lymphoid*, *erythroid* and probably *thrombocyte* precursor cells start in intra embryonic mesenchyme in the para-aortic region around 2-3 days of *embryonation* Primitive lymphoid stem cells travel to the yolk sac between days 2 and 7 of *embryonation* and are first found in yolk sac on days 7. The earliest the *prebursal* and *prethymic* stems are detected is day 5, but it is speculated that they are present starting days [15].

The thymus is populated in three waves (chicken and quail) by extrinsic stem cell from the yolk sack (probably also the spleen and by *embriyonatoin* day four, the thoracic aorta as well may directly contribute [15]. The avian liver is not an important transient site for prethymic stem cell as in mammals. The liver is mainly for extrahematopoiesis and prebursal stem cell transient [2]. The bursa is first observed as an outgrowth of the uroanal membrane (Based on the posterior cloaca) at 3-4 days of *embriyonation* [16]. Epithelial cell in the bursa attract

bursal stem cell to the bursa, entrance to the bursa is regulated at the level of the endothelial cell of the bursa microvasculature. Epithelial cell retain the ability to attract lymphocytes even after involution [3].

Bursal stem cells from the yolk sack or other site of hematopoiesis such as the spleen, liver, bone marrow and blood seed the bursa starting around 7-10 though 15 days of *embryonation*. Those that remain outside of the bursa (Most of the population) undergo apoptosis lymphocytes from the bursa seed secondary lymphoid organs just prior to and post hatch. The bursa is required for generation of anti-body diversity and functional maturation of the B cell and the thymus is required for T cell maturation. Major histocompatibility complex (MHC) dosage appears to modulate the cellularity of the bursa and thymus [17].

Chicken have been produced that have three and four copies of the MCH micro chromosome. These chickens have decreased bursal thymus and body weight. Both the bursa and thymus involve at sexual maturity and it is unknown where the post bursal and thymic stem cell they presumably maintain the T and B cell after involution are located [2]. There are three populations of B lymphocytes in the periphery. A cell surface antigen, IL2 which is on all precursor cell, is help to sort three populations (1) short life span (3 days): the peripheral blood lymphocyte concentration is 60%, comprise 60-95% of the bursal immigrants do not divide in periphery, come from the cortex, have high level of IL2 which decrease with age, disappear after involution. (2) Long lived (2 weeks): 35% of the peripheral blood lymphocytes do not divide periphery, 5-10% of the bursal immigrants. (3) Indefinite life span (Unable to document through surgical bursectomy): divide rapidly, life span of individual cells is probably short but cells are always being replaced, population in blood increase with time "postbursal stem cells" [16].

**Hematopoietic Growth - Factors:** Hematopoietic growth factors are components that required for the survival and proliferation of hematopoietic cells at all stages of development. The identification and description of avian cytokines and lymphocytes has expanded exponentially in recent year [18]. Due to the general lack of homology between avian and mammalian cytokines, use of mammalian monoclonal antibodies for identification of avian cytokines results in limited information. Most of the research has been done with chickens, ducks and turkeys. To date the following cytokines have been either fully or partially characterized, the following cytokines have been

either fully or partially characterized in avian species *erythropoietin*, interleukin (IL-1, IL-2, IL-6, IL-8, IL-10, IL-12, IL-16, IL-18), granulocyte colony stimulating factors, *thrombocyte* inhibitory factor and tumor necrosis factor [2].

**Erythrocytes:** Avian erythrocytes are oval, nucleated cells with round immature form. Mature chicken and turkey erythrocytes found in the peripheral blood are large elliptical cells measuring approximately 12x6µm they have homogenous eosinophilic cytoplasm and a central round to oval nucleus with a condensed chromatin pattern. Because *erythropoiesis* is intra vascular or *intrasinusoidal*, occasional *rubricates* can be found in the peripheral blood of health birds [2]. Erythrocytes are the most common cell in avian (And other non-mammalian) blood, in all non-mammals, the erythrocyte of the circulation is a true cell; complete with nucleus. As in mammals, its function is to carry oxygen to the tissue [19].

The percentage of *reticulocytes* seen in the peripheral blood of normal chickens and turkeys is somewhat higher than in most mammals. *Polychromesia* is more prominent in younger birds, but typically does not exceed 5% common artifactual abnormalities in erythrocyte morphology include cytoplasmic refractile vacuoles, smudge cells and various morphologies includes by stretching cells while preparing the smear such as splinding, bilobbed nuclei, bare nuclei and *erythroplastids* (A nucleate fragments of erythrocyte c cytoplasm)[2].

**Granulocytes:** As in other avian species, the peripheral blood of chickens and turkeys contains heterophils, eosinophils and basophiles and they arise from a common precursor in the bone marrow [2].

**Heterophils:** The avian *heterophils* which is mostly compared to that of the *neutrophils* in mammals is the most frequently seen granulocyte in all species. As with *neutrophil*, the *heterophils* are instrumental in body defenses with incredibly large number available as well as their ability to phagocytes foreign bodies' bacteria [19]. However, there are differences in granule contents and response to some stimuli. It has been speculated that avian *heterophile* either do not contain or contain minimal quantities of species *lysosome*. *Heterophils* lack *myeloperoxidase* and alkaline *phosphatase*. Chicken and turkey *heterophils*, unlike *neutrophils*, do not respond to stimulation by *formylmethionyl-leucylphenylalanine* (FMLP) and then production of oxygen radicals is lower than that of mammalian *neutrophils* [1].

The avian *neutrophils* are circular shaped with a blobbed nucleus. This may or may not be very visible through the rod sizes and shaped granule contained in the colorless to faint blue cytoplasm., depending on species there can be quite variation of rod sizes and shape and also whether can be quite a variation of rod sizes and shaped and also whether the cytoplasm is colorless or faint blue. Using the recommended stain, the rods should stain a muddy pink in most species and the cytoplasm will stain colorless to uneven variations of pink in case of moderate to heavy degeneration [19].

*Heterophils* appear to be the first line of defense against bacterial pneumonia, although the ability to generate oxidative metabolites is lower in *heterophils* derived from the respiratory tract than in *heterophils* from the peripheral blood. Defensins are antimicrobial proteins stored in the *lysosome* like granules of *heterophils*, macrophages and epithelial cells, originally termed as "*Lysosomal cationic proteins*" in the *neutrophils* of rabbits, there are two types, alpha and beta in man and a third type, theta found only in rhesus monkeys. Only the betas have been found so far in birds. Gallinacin-3 has been identified in chickens and gallopvin-1 in turkeys [20].

**Eosinophils:** Eosinophils are round to irregular shaped and are approximately 12 µm in diameter. They have a lobed nucleus and light blue cytoplasm with eosinophilic round to oval granules that stain brighter than those of *heterophils* and lack of central body. Immature *heterophils* have round granules and could be confused with eosinophils. Cytochemical stains can distinguish between the two types of cells. *Eosinophils* are positive for *peroxidase* and acid phosphatase activity and Sudan black B while *heterophils* are negative [1].

The staining characterized of this cell appear hinge on the type of stain used and species of birds we are dealing with. In labs the granules of the African grey, love birds and raptors for example, do frequently stain a faint pink color. (This color is not comparable to the *heterophils* which is good) with this species. The cytoplasm will often be abundantly filled with granules, causing difficulty with identification. Other species such as cockatoos, Amazons seems to stain colorless faint blue, but are recognizable due to the shape of their granules. With both groups the nuclei stain much more intensely than the *heterophil* nuclei [19]. Eosinophils are typically associated with allergic reaction and parasitic infections and rarely seen in high number except in raptor species [6].

Table 1: The Erythrocyte maturation sequences

Cell maturation stage	Description
• Rubriblast	• Large cell, central round nuclei, granular chromatin, prominent nucleoli, very basophilic cytoplasm with mitochondrial spaces.
• Prorubricyte	• Nucleoli and mitochondria spaces in apparent.
• Basophilic rubricyte	• Clumped chromatin pattern, very basophilic cytoplasm.
• Early Polychromatic rubricyte	• Cytoplasm becoming grayish, indicating hemoglobin production, chromatin more clumped, smaller nucleus.
• Polychromatic rubricyte	• More grayish to eosinophilic cytoplasm clumped chromatin, small round nucleus.
• Late polychromatic rubricyte	• Similar to mature erythrocytes but slightly large with a more basophilic cytoplasm; should be 1-5% of circulating erythrocytes.
• Polychromatic erythrocyte mature erythrocyte	• Homogeneous eosinophilic cytoplasm, oval cell with central oval nucleus and considered chromatin pattern.

Source: Wakenell [2]

Table 2: The granulocyte series maturation sequence

Cell maturation stage	Description
Myeloblast	Large, round cell with rim of lightly basophilic cytoplasm around a large nucleus with a delicate chromatin pattern and nucleoli.
Promyelocyte	Light blue cytoplasm, eccentric nucleus with delicate chromatin pattern, primary granules in cytoplasm are orange spheres, heterophil and basophil promyelocytes also have magenta granules and rings (smaller in basophil promyelocyte).
Myelocyte	Smaller, more condensed nucleus, contain less than half the definitive number of specific or secondary granules and still retain magenta granules and rings (except eosinophil myelocytes)
Metamyelocyte	Smaller, slightly indented nucleus, more than half the number of definitive granules.
Band Mature	Similar, to mature cell without nuclear lobes, nucleus is elongate to U-shaped, rare to see bands in circulation in avian blood, usually see mature granulocytes only.
Eosinophil	Round, similar to heterophil but granules more brightly eosinophilic (more arginine) and without central refractile body' cytoplasm usually pale blue.
Mature Basophil	Round cell with a round central nucleus; deeply basophilic cytoplasmic granules that may partially obscure the nucleus and dissolve or coalesce in alcohol solubilized stains like Wright's.

Source: Wakenell [2]

**Basophiles:** The basophil is a round cell with deeply basophilic granules in the cytoplasm and a round light blue, central, non-indented nucleus. It resembles the mammalian mast cell, birds appear to have more mast cells and basophils than mammals. The basophil measures approximately 12µm in diameter and the granules often partially obscure the nucleus. These granules may dissolve or condense in wrights stains [2]

*Basophils* are one of the first leukocytes to enter tissue as part of the early inflammatory response in birds and the cutaneous basophile response in birds and the cutaneous basophile region response can be measured to help assess immune competence [21].

The easiest granulocyte to recognize tend to be *basophils*, when stained, the granules of the *basophile* become a redish-purple color. The *basophilic* granules are usually much smaller than the granules of *eosinophil*, the cytoplasm stains a colorless to a light purple, red color and the nucleus doesn't stain as intensely as the *eosinophil*. In case of extreme toxicity, *heterophils* have been found to have very *basophilic* granules also. To avoid counting a toxic *heterophils*, notice the darker staining nucleus of the *basophile* and the absence of

cytoplasmic ridges. Usually the cell with very basophilic granules will be a basophile. *Basophiles* are seen in high percentages with finches, canaries. Smaller birds and are most of the time associated with chronic, long term illness [19].

**Agranulocytes (Mononuclear Cells):** Lymphocyte and monocytes comprise this group of white blood cell in exotics as well as mammals. Since these cells do not have granules, they can be difficult to differentiate, especially if the stain used does not give good clarity and definition of the cell nucleus [19].

**Monocytes:** Chicken and turkey monocytes are usually the largest leukocytes (Approximately 14µm in diameter) and need to differentiate from large lymphocytes. Monocytes are round cell usually had indented nuclei and abundant pale, vacuolated blue-grey cytoplasm. The cytoplasm contains fine azurophilic granules. The monocyte is a pleomorphic cell and can be difficult to identify. It is important to be consistent with lymphocyte classification to aid in differentiation from monocytes. Lymphocytes are round and generally have less

cytoplasm than monocytes, size is dependent on activation state in chickens and blood monocytes cannot be differentiated from lymphocytes solely on the basis of size [2].

Most of the monocyte are the least numerous and the largest white blood cells, it can range from bean-shaped to a very indefinite shape with a fine, lack Reticular chromatin pattern. Chicken and turkey monocytes are similar to the mammalian cells. Monocytes from chicken and turkeys are capable of oxidative as well as phagocytosis and killing. Heterophils from chickens and turkeys have greater phagocytic activity and killing ability than macrophage [19].

**Lymphocytes:** Lymphocytes are the predominant leukocyte in the peripheral blood of chicken and turkeys. Both small and medium lymphocytes normally occur. The small lymphocytes are round with a round nucleus, clumped chromatin, high nuclear: cytoplasm (N: C) ratio and a rim or small basophilic cytoplasm. Occasionally cytoplasm of small lymphocytes may only be seen as cytoplasm projection and they may confuse with thrombocytes. Thrombocytes distinguish by their clear cytoplasm then often mold around cells. Lymphocytes may have nuclear indentation and sometimes larger lymphocytes may have more angular nuclei or nuclei with flattened size [2].

Reactive lymphocytes can be seen in peripheral blood. Cell size is increased and the cytoplasm is deeply basophilic sometimes with clear pronuclear area (Golgi) plasma cells may only rarely be found in peripheral blood. Reactive lymphocytes may resemble rubricytes. However, rubricytes have a lower N: C (Nuclear to cytoplasm) ratio and are typically seen in peripheral blood with polychromatophilic erythrocytes in regenerative response [22]. The presence of eosinophilic (Azurophilic) granules peripheral blood Lymphocytes is rare and its significance is unknown. These cells are readily distinguished from monocytes, because the lymphocytes azurophilic granules are larger and more intensely staining on the dust like granules of monocytes [2]. There are some diseases state that can cause a severe Lymphocytes increase meaning more lymph's than hats would be seen. In some case spatially in amazons lymphocytes count as high as 85% have been noted but after treatment count remained from 60-65% [19].

**Thrombocytes:** Chicken and turkey thrombocytes are round to slightly oval cells with a round nucleus in the

center of a clear cytoplasm. Thrombocytes can be confused with small lymphocytes in tissue samples and blood smear. Monoclonal antibodies against chicken thrombocytes have been developed and can aid in identification [23]. Avian thrombocytes contain azurophilic cytoplasm, granules, Stain periodic acid Schiff (PAS) and Grimelius positive clump readily and produce little thromboplastin. It is likely they are capable of phagocytosis, although definitive proof has not been obtained [24].

Avian thrombocytes has light blue to colorless cytoplasm containing approximately two to four reddish granules usually at one end of the cell (called the pole). The nucleus stain very darkly and rarely has visible clumps [19].

*Avian thrombocytes* although their appearance is much different from mammal platelet, they perform primarily the same function as their mammalian counterpart. Other *thrombocytes* function have been speculated on, such as their possessing the ability to phagocytes foreign material in over whelming situation, their response (reaction) to outside stimuli and lastly, the possibility that they might be able to replace RBCS, in case of extreme anemia [19].

#### **Normal Reference Intervals for Chicken and Turkeys:**

Total erythrocytes numbers are approximately  $3 \times 10^6/\mu\text{l}$  or a PCV of 25-45%. chicken typically have a lower PCV (as low as 24%) that increase with age. The MCV has been reported in the range 90-140 fl [22]. Chick tends to have fewer, larger erythrocytes. Hemoglobin concentration of chicken and turkeys have been reported from 8.6 to 15.2 gm/dl. Variation in erythrocytes parameters may reflect different line of chicken and turkeys and different method of determination. Erythrocyte life span is 28-35 days in chicken [6].

Early data showed PCV to be lower heavy type (30.1-30.9) than in white longhorn types (31.9-33.9) chickens. More recent hematology data shown are shown in the table below. Mean hematocrit from specific pathogen free white leghorn chicken of 5-42 days of age range from 32.7%-36.6% respectively [22].

Limited hematological data are available from domestic turkeys. As in chickens; studies have shown that differences exist in hematologic parameters of closed genetic lines and different commercial lines of turkeys, large body birds bred for meat production had lower lymphocytes counts, higher heterophils counts and higher total erythrocyte counts than line bred for egg production [7].

Table 3: The monocyte maturation sequence.

Cell maturation stage	Description
Monoblast	A poorly defined cell, probably indistinguishable from the myeloblast Large cells with abundant, clear blue cytoplasm, round nucleus, reticular chromatin
Early Promonocyte	Round, eccentric nucleus with granular basophilic cytoplasm, sparse eosinophilic granules
Late Promonocyte	Large and irregular with a round to bilobed nucleus, fine chromatin pattern, abundant blue –gray
Mature Monocyte	granular cytoplasm and occasional cytoplasmic vacuoles and/or fine dust like eosinophilic granules

Source:Wakenell [2]

Table 4: The Thrombocyte maturation sequence

Cell maturation stage	Description
Thromboplastic	Large, amoeboid cells with a rim of very basophilic cytoplasm around a large nucleus with punctate chromatin (chromatin is not coarse as in the rubriblast), nucleolus not always apparent, some clear vacuoles within cytoplasm.
Early immature Thrombocyte	Slightly lower N:C than thromboplastic, more cytoplasmic vacuoles evident; chromatin becomes clumped.

Source:Wakenell [2]

Table 5: Reference intervals for leucocytes from health 22-24 week old male broiler type chickens.

S.No	Cell type	Reference interval
1	Leukocytes	7940-24280
2	heterophils	1703-9746
3	Lymphocytes	2639-10294
4	monocytes	544-4123
5	Eosinophils	0-346
6	Basophils	382-2499

Source:Bounous and Stedman [22]

Table 6: Reference intervals for healthy wild turkey poults.

S.No	Parameters	Reference interval
1	PCV (%)	30-41.5
2	Leukocytes	13917-46609
3	Heterophils	4046-24231
4	Lymphocytes	4156-31138
5	Monocytes	0-3756
6	Eosinophils	0-420
7	Basophils	23-2039

Source: Bounous and Stedman [22]

or deficient production of one of the hematopoietic component; these problems can be approached by identifying the primary hematopoietic component that is affected.

**Recommendations:** Depend on the above conclusion the following recommendations are forwarded.

- Hematology should be incorporated in routine testing done by practitioners at their clinic.
- Blood smear should be made immediately after blood collection even if the rest of blood evaluation cannot be performed.
- The clinician must put time and effort in to knowing the fairly sampling techniques, as well as differentiating the normal morphology of blood cells.
- Clinicians have to have a guideline that shows a normal reference interval of hematopoietic component.

## CONCLUSIONS

In conclusion clinical hematology is qualitative and quantitative assessment of blood and other blood component for the treatment of clinical patient. This includes everything from managing the patient with genetic anemia to the treatment of patient with acquired blood born disease and other organ disorder. In comparison to mammals' birds do not have bilirubin and bile acid their erythrocytes are oval, nucleated cells with round immature form. Hematopoietic abnormalities arise due to either excessive

## REFERENCES

1. Ramirez, P., 2004. Introduction to hematology. [Accessed at: <http://www.academia.edu>]. [Viewed on April, 2013].
2. Wakenell, P.S., 2010. Hematology of chicken and turkey. In: D.S. Weiss and K.J. Wardrop, (eds): Schalm's veterinary hematology. 6<sup>th</sup> ed. Blackwell Publishing, pp: 958-966.
3. Zekarias, B., A. Terhurne and W.M. Landman, 2002. Immunological basis of differences in disease resistant in chicken. Vet. Res., 33: 109-110.

4. Hendrix, D.J., 2006. Laboratory manual for vet technician. [Accessed at: <http://www.vet med.ws u edu /client ED/; lab asp>] [Viewed on April, 2013].
5. Cooper, J.E., 2001. Bird of prey health and disease. 3<sup>rd</sup> ed., pp: 57-59.
6. Samour, J., 2004. Avian medicine. 1st ed. Saudi Arabia, pp: 28-40.
7. Campbel, T.W., 2000. Normal hematology of psittacine. In: B.F. Field Man, J.K. Zink and N.K. Jain, (Eds): Schalm's Veterinary hematology. 5<sup>th</sup> ed. Philadelphia: Lea, pp: 1155-1160.
8. Guzman, D.S., M.A. Mitchel and S.D. Gaunt, 2008. Comparison of hematologic values in blood sample with heparino or dipotassium ethylene diamine tetra acetic acid anticoagulant in istopanoilan Amazon parrots. Avian Med Surgery, 22: 157-160.
9. Pierson, F.W., 2000. Avian hematology. In: field man, B.F., Zink, J.K., Jian, N.C., (eds): Schalm's veterinary hematology. 5<sup>th</sup> ed. Philapidia: Lea and Fibiger, pp: 1145-1146.
10. Kass, L., G.L. Harrison and C. Lindheimer, 2002. Anew stain for identification of avian leukocytes. Biotechnic Histochem, 77: 201-205.
11. Lillihook, I., H. Wall and R. Tauson, 2004. Deferential leukocyte counts determines in chicken blood using the cell –Dyn350. Vet Clinical Pathology, 33: 133-135.
12. Moritimo, T., A. Minami and Y. Inoune, 2002. New method for counting of quill leukocyte by flow cytometry. J. Vet. Med. Sci., 64: 1149-1151.
13. Zoonli, C., 1995. Development biology of hematopoiesis. Blood, 86: 2676-2777.
14. Pike, K.A., E.M. Biag and M.J. Ratclif, 2004. The avian B-cell receptor complex distinct role. Ig alpha and Ig beta in B- Cell Development, 197: 10-13.
15. Dunon, D. and B. Imhoof, 2000. The role of cell traffic in the emergence of the T –lymphoid system. Seminimmunol, 12: 429-432.
16. Ret cliff, M.J., 2006. Antibodies, immunoglobulin gene and the bursa of fabricus in chicken B cell development. Deve Comp Immunol., 30: 101-114.
17. Delany, C., O. Gandrillon and S. Gonin-Giraud, 2008. Stem cell antigen 2: a new gene involved in the self-renewal of erythroid progenitors. Cell Proliferation, 41: 726-728.
18. Giasanti, F., M. Giardi and O.F. Botti, 2006. Avian cytokines: An over View. Currpharmaceut Des., 12: 3083-3099.
19. Rosalie, S. H., 1991. Structure and function of the chicken spleen. Res. Immunol., 142: 352-354.
20. Zhao, C., T. Nguyen and L. Liu, 2001. Gallanacin -3, an inducible epithelial beta defensein the chicken. Infect Immunol., 69: 2684-2691.
21. Koutsos, E.A., J.C. Lopez and K.S. Klasing, 2007. maternal and dietary carotinoids enter actively affect cutaneus basophile response in growing chicken. Comp Biochemphysiol. B, 147: 87-90.
22. Bounous, D. and N.L. Stedman, 2000. Normal avian hematology: chicken and turkey. In: field man, B.F., J.G. Zinkl and N.C. Jain 1998 (eds): Schalms veterinary hematology. 5<sup>th</sup> ed. Philadelphia: Lea and Fibiger, 364: 1148-1150.
23. Horiuchi, H., K. Tanaka and A. Shigeta, 2004. Monoclonal antibody against chicken thrombocytes reacts with the cell of thrombolytic lineage. Vet. Med. Sci., 66: 243-250.
24. Meseguer, J., M. Esteban and A. Rodriguez, 2002. Are thrombocyte and platelets true phagocytes? Microsc Res. Tech., 57: 491-492.